(-18 ± 6ms vs. -29 ± 3ms, p=0.07). However, the GPS-induced CV slowing was abolished by H9335 (97 ± 14cm/sec, vs. 103 ± 15cm/sec at baseline and 101 ± 16cm/sec after recovery, p=0.23). The effects of endogenous VIP were eliminated by atropine during GPS, indicating VIP release from intrinsic cardiac neurons may be mediated by muscarinic recpetors. Conclusion: Neuronally released VIP could contribute vagal effects and is primarily responsible for CV slowing during GPS. Neuronal release of VIP is likely mediated by muscarinic receptors in the intrinsic cardiac neurons.

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Hearts of K Channel-Interacting Protein 2 Deficient Mice have Prolonged Action Potential Duration, and Reduced Outward Potassium Currents that are further reduced by Heart Failure

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Cardiac myocytes exhibit a fast recovering transient outward potassium current ($I_{to,f}$) that is conducted by the channel proteins K_v4 + KChIP2. Heart failure (HF) is associated with a reduction of outward K^+-currents and a downregulation of KChIP2. In this study, we investigated the electrophysiological role of KChIP2 in mice with HF.

HF induced by transverse aortic constriction was defined as an ejection fraction below 50% evaluated by ultrasound. Action potentials were recorded by floating micro electrodes from the left ventricle in *ex vivo* perfused hearts. Whole-cell currents were recorded from disaggregated left ventricular cardiomyocytes. We found an equal reduction in the ejection fractions of wild-type (WT) and KChIP2$^{-/-}$ mouse hearts by transverse aortic constriction. Left ventricular action potential durations were prolonged by 32% in KChIP2$^{-/-}$ vs. WT at 90% repolarization (APD_{90}) during 200 ms pacing intervals ($P<0.05$). Peak K^+-current density was 18% lower in KChIP2$^{-/-}$ vs. WT ($P<0.05$). Induction of HF reduced peak K^+-current density by 38% in KChIP2$^{-/-}$ and by 24% in WT ($P<0.05$ for both). K^+-current recovery from inactivation was delayed in KChIP2$^{-/-}$ vs. WT, and further delayed by HF in KChIP2$^{-/-}$ but remained unchanged in WT.

In conclusion, HF reduced K^+-currents in WT, but did not abolish the KChIP2-dependent fast recovering current component. KChIP2$^{-/-}$ mice lack the fast recovering K^+-current ($I_{to,f}$) leading to prolonged left ventricular action potentials. HF in KChIP2$^{-/-}$ mice induced a further loss of K^+-currents and an additional delay of recovery from inactivation, suggesting a reduction of non-$I_{to,f}$ K^+-currents contributing to the electrophysiological changes in mouse HF.

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Low and High pH Gating of Connexin43 Channels

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Intracellular pH (pH_i) regulation in myocardial tissue is crucial to preserving cardiac function. H^+-ions can flow passively at high rates through connexin gap-junctions to neighbouring cells, helping to maintain pH_i-uniformity in multicellular tissue. In ventricular myocytes, this form of spatial H^+-dissipation is regulated by pH_i at the junction.

We have studied the response of connexin43, the main ventricular junctional channel-isoform, to high and low pH_i. Transjunctional conductance (Gj) was measured by double whole-cell voltage clamp in HeLa or N2A cell-pairs transfected with connexin43. pH_i was manipulated with 80mM acetate or 20mM trimethylamine in the superfusate. Acid (<6.7) and alkaline (~7.5) pH_i reversibly reduced (<5min) cell-to-cell Gj by $35 \pm 4\%$ and $56 \pm 3\%$ respectively (n=4-5), relative to the values at resting pH_i (~7.0). Acute electrical uncoupling at high and low pH_i occurred with no change in single channel conductance (2mM halothane; $122 \pm$ pS, n=250), suggesting a channel-gating mechanism. Cells expressing a truncated C-terminus mutant of connexin43, showed no conductance response to pH_i-changes.

Using a different approach, we measured apparent junctional H^+-permeability (P_H^{app}) through connexin43. One cell of a pair was acid-loaded by photolytically uncaging H^+ from an intracellular donor compound (nitrobenzaldehyde), while pH_i was confocally imaged in both cells (SNARF-1). Cell-to-cell H^+-diffusion was inhibited by β-glycerrhetinic acid (connexin-blocker, 60μM) and was negligible in wild type cell-pairs. In HCO_3^--free buffer, presetting pH_i to 6.6 or 7.3, reduced P_H^{app} by $81 \pm 6\%$ and $76 \pm 14\%$, respectively (n=5-19). P_H^{app} returned to initial values when pH_i was allowed to recover. The presence of $5\%CO_2/22mMHCO_3^-$ in the perfusate doubled P_H^{app}.

In conclusion, connexin43 gap-junctions exhibit reversible block at both acid and alkaline pH_i, possibly by a gating mechanism involving the cytoplasmic C-terminal tail. This suggests a key role for connexin43-channels in coordinating cell-to-cell electrical coupling and spatial pH_i regulation during metabolic stress.

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Effect of Acute Hyperglycemia on Cardiac Conduction

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Diabetes is associated with a relatively high prevalence of sudden cardiac death but the underlying mechanism is not well understood. In experimental diabetes, cardiac expression of connexin43 is reduced. Downregulation of connexin43 is linked to a slowing of cardiac conduction which increases the risk of cardiac arrhythmias and thereby sudden cardiac death. It is known that hyperglycemia causes downregulation of connexin43 but it is unknown to which extend this occurs in whole hearts. Here, we test the hypothesis that acute hyperglycemia leads to slowing of cardiac conduction through downregulation of connexin43 in whole hearts.

We measured ventricular conduction velocity in Langendorff perfused guinea pig hearts using a multi-electrode array under normo- and hyperglycemic conditions (5.5 and 30mM glucose respectively) for up to 4 hrs. In addition, one group of hearts was perfused with a mannitol solution as a control for changes in osmolarity. Expression of connexin43 was quantified by western blotting. In the control group, longitudinal conduction velocity remained stable over 4 hrs (63 cm/s) while transverse conduction decreased slightly (from 27 to 25 cm/s). Similar results were obtained from both the hyperglycemia and mannitol groups suggesting that conduction was unaffected by changes in glucose concentration and osmolarity. Accordingly, no differences were observed in the protein levels of connexin43 between the groups supporting the functional results.

In conclusion, acute exposure to hyperglycemia does not appear to have any significant effects on cardiac conduction and does not modulate the expression of connexin43 in guinea pig hearts.

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Manipulability of β-Adrenergic Responsiveness in Adult Guniea-Pig Cardiomyocyte Cultures

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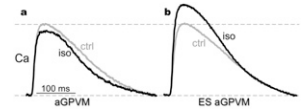
β-adrenergic stimulation of cardiomyocytes induces vital upregulation of both chronotropy (heart rate) and ionotropy (contractility). Progressive attenuation of this regulation constitutes critical pathogenic and therapeutic dimensions of heart failure (*Gene Ther***19**:686). Yet, key features of this pathway and its dysfunction in disease remain unclear. Highly valuable would be a long-term cultured myocyte system that emulates the strong-to-weak progression of β-adrenergic responsiveness between normal and diseased states, while permitting ready manipulation and measurement of underlying molecular events. Here, we used improved culture protocols to produce functional monolayers of adult guinea-pig ventricular myocytes (aGPVMs) that can be maintained for several weeks. At baseline, these monolayers support propagated electrical activity and exhibit monophasic restitution of action-potential duration and conduction velocity. Intriguingly, β-adrenergic stimulation (0.5 μM isoproterenol, iso) upregulates chronotropy without change in inotropy (**a**, Ca^{2+} transients), indicating selective regression of adrenergic signaling. By contrast, after several-day electrical stimulation of cultures, β-adrenergic stimulation not only augments chronotropy, but robustly enhances inotropy (**b**). Thus we have generated a cultured myocyte system whose β-adrenergic responsiveness can be easily manipulated, offering a valuable platform for clarifying long elusive features of adrenergic signaling and its plasticity in disease.



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Involvement of PDE2 in a Localized Regulation of the L-Type Ca^{2+} Channels by Progesterone

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We have demonstrated that a nitric oxide production induced by stimulation of progesterone (P_4) receptor suppresses cAMP-stimulated L-type Ca^{2+} currents ($I_{Ca,L}$) through an increase in cytosolic cGMP, which may be related to the